investigators also have the ability to vary which G-protein isoform to use in order to look for additional levels of specific regulation between the channel types. One potential problem here is that with excision diffusible factors are more likely to leave the channel complex and alter the results. Some of these will be addressed in later parts of this project (PIP2, PKC, etc.) but there may be others that could confound the results.

In the 3rd part of Aim 1 the applicant proposes to apply signaling molecules to the cytoplasmic surface of atrial patches to further investigate differential signaling to the 2 channels. These are chosen based on previous data generated in the applicant's laboratory: catalytic subunit of PKA and PP1. It is not entirely clear if these are the only proteins to be used here and what other cofactors will be used (Mg^{2+}, ATP, Na^{+}, etc.). Moreover, specific interpretation of possible results is not discussed. In summary of Aim 1 the applicant states that the goals of these experiments are to determine the "dynamic and spatial aspects" of scGIRK regulation. The dynamic aspect does seem to be covered here but it is not clear to me how spatial (or in what sense spatial) aspect is investigated.

For the 2nd aim the applicant proposes to examine the role of PIP2 and PKC in the regulation of GIRK channels from the atrial. This is a topic that has been investigated by several other labs with some controversy however there seem to be some convergence of opinion recently: Gq-coupled receptors reduce GIRK activity by consuming PIP2, while PKC (especially the delta isoform) appears to sensitize the channels to changing PIP2 concentrations. What this aim proposes that is new is to later PIP2 and/or recombinant PKC isoforms to the cytoplasmic face of the atrial patches and subsequently assays alterations in $G_{beta/gamma}$ binding. The strength of this is that it is being tested in heart tissue and that single channel analyses will allow more precise measurements of channel-G-protein interactions. If carefully executed with each of the cardiac expressed PKC isoforms and with different concentrations of PIP2 (high and low) these studies could help dissect out the actual pathways that are regulating these channels. Additional methods of altering PIP2 concentrations independent of PLC activation that should be considered include the use of neutralizing PIP2 antibody or recombinant Inp54p, a yeast inositol polyphosphate 5-phosphatase (Raucher *et al., Cell* 100, 221 (2000)).

The applicant makes the claim that these experiments will allow her to make the "molecular identity" of the scGIRK channels. This reviewer is not sure how this can be accomplished wit the described experiments. Adding PKC isoforms may or may not alter channel behavior differently for different isoforms and different between scGIRK and GIRK1/4 but it does not tell you that they are really different proteins, much less their identity. Differences in response to PKC could be due to direct channel phosphorylation on different sites or on intermediary proteins.

Innovation: This proposal represents application of standard technology to native tissues to address some unresolved questions regarding IKACh. This in it self, is not entirely innovative. The approach of using their precise single channel gaiting analyses brings something new and potentially useful to the story. The investigation of scGIRK as a possibly differentially regulated GIRK is a new idea and possibly important.

Investigators: Dr. Ivanova-Nikolova finished her training in 1996 and took an Assistant Professor position at East Carolina University in 1999. There have been 2 papers in peer-reviewed journals published during this time and another in revision at JBC. The applicant has ample experience at performing all the experiments proposed.

Environment: The applicant's laboratory is equipped for basic electrophysiological and biochemical studies. There is a research technician, Dr. Nikolova, who is an experienced biochemist and has been working with the applicant for several years in the area of GIRK channel research. East Carolina University Medical School does not have a reputation as an institution with a long track record of biomedical research. The general intellectual environment for this type of work is not the best. Nevertheless, the applicant has continued to publish careful and high-quality research papers on a regular basis, attesting to the adequacy of the environment for this particular scientist.